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Anandamide hydrolysis: a new target for anti-anxiety drugs?

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The major psychoactive constituent of cannabis, Δ^9-tetrahydrocannabinol, affects emotional states in humans and laboratory animals by activating brain cannabinoid receptors. A primary endogenous ligand of these receptors is anandamide, the amide of arachidonic acid with ethanolamine. Anandamide is released in selected regions of the brain and is deactivated through a two-step process consisting of transport into cells followed by intracellular hydrolysis. Pharmacological blockade of the enzyme fatty acid amide hydrolase (FAAH), which is responsible for intracellular anandamide degradation, produces anxiolytic-like effects in rats without causing the wide spectrum of behavioral responses typical of direct-acting cannabinoid agonists. These findings suggest that anandamide contributes to the regulation of emotion and anxiety, and that FAAH might be the target for a novel class of anxiolytic drugs.

Pharmacological studies of anxiety disorders have been traditionally focused on the γ-aminobutyric acid (GABA)A–benzodiazepine receptor complex but several additional neurotransmitter systems have recently emerged, which might play important roles in these conditions [1]. A case in point is the endocannabinoid system, which is constituted by the cannabinoid receptors – the molecular target of Δ^9-tetrahydrocannabinol (Δ^9-THC) in cannabis – and their attending family of lipid-derived ligands [2].

Retrospective studies in cannabis users [3,4], small clinical trials [5], as well as animal experiments [6] have drawn a complex, often contradictory picture of the impact of cannabis on emotions. In humans, the drug produces a variety of subjective effects, which can range from relaxation and euphoria to anxiety and acute panic attacks [7]. Similarly, in animals, Δ^9-THC and other cannabinoid agonists can exert both anxiolytic-like and anxiogenic-like actions, depending on a set of variables – such as genetic background [8,9], drug dose [10,11] and environmental context [12] – which remain poorly understood.

How can we explain this contradictory scenario? In part, it might be due to the presence of cannabinoid receptors in brain regions that serve different functions in the regulation of emotions [2,13]. Another reason might be the cellular localization of CB_1, the main cannabinoid receptor subtype in the central nervous system (CNS). In the forebrain, CB_1 is primarily localized to axon terminals of GABAergic interneurons, where its primary effect is to decrease GABA release [2]. In some of these areas, CB_1 (or a functionally related isoform) is also found on glutamatergic nerve terminals and its activation inhibits glutamate release [14]. As a result of this distribution, CB_1 activation might have dramatically different behavioral consequences, depending on the balance of its effects on GABAergic and glutamatergic transmission within a neural network. Therefore, the question arises as to what, if any, is the role of the endocannabinoid system in the regulation of emotion. The fact that pharmacological blockade or genetic ablation of CB_1 produces anxiety-like states in rats and mice suggests the existence of an intrinsic anxiolytic tone mediated by endogenous cannabinoid (endocannabinoid) ligands [15,16] (but for contrasting results see [17]).

Endogenous cannabinoids

Best studied among the endocannabinoids are the lipid derivatives anandamide (arachidonoylethanolamide) and 2-arachidonoylglycerol (2-AG) [2]. The presence of an arachidonic acid chain gives to both compounds a structure that superficially resembles those of the eicosanoids (e.g. prostaglandins, leukotrienes; Figure 1). Yet, the mechanism of endocannabinoid synthesis differs from those of the eicosanoids in that it does not involve metabolism via lipoxygenase, cyclo-oxygenase or cytochrome P450 enzymes. Moreover, anandamide and 2-AG differ from classical and peptide neurotransmitters because
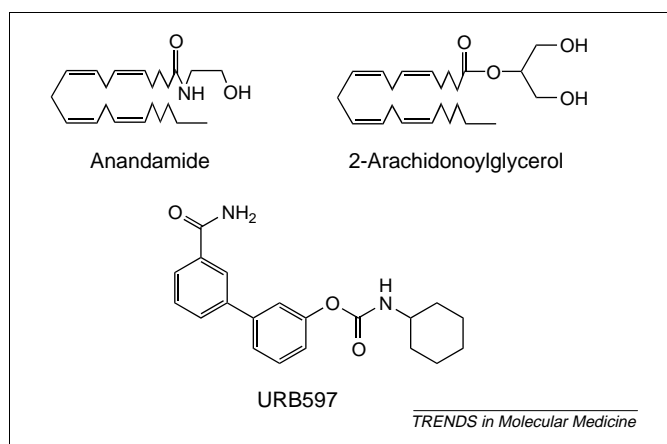
Figure 1. Chemical structures of the endocannabinoids anandamide and 2-arachidonoylglycerol (2-AG), and of the fatty acid amide hydrolase inhibitor URB597.

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their release from neurons is not mediated by exocytosis of storage vesicles. Rather, current models suggest that anandamide and 2-AG are produced upon demand through activity-dependent cleavage of membrane lipid precursors and are released from cells immediately after their production [18,19]. These properties underpin the suggested role of anandamide and 2-AG as activity-dependent modulators of synaptic function [20–22].

Despite their similarities in chemical structure, anandamide and 2-AG are synthesized through different biochemical pathways. The former is produced via the hydrolysis of an N-acylated species of phosphatidyl-ethanolamine (PtdEtn), N-arachidonoyl-PtdEtn, which requires the activity of an unknown phospholipase D [18]. 2-AG is probably generated via an enzymatic cascade similar to the one that catalyzes the formation of the second messengers inositol-(1,4,5)-trisphosphate and 1,2-diacylglycerol (1,2-DAG): a phospholipase acting on membrane phosphoinositides generates 1,2-DAG, which is then converted to 2-AG by a DAG-lipase activity [19].

After release, anandamide and 2-AG are rapidly eliminated through two separate mechanisms, which resemble those involved in the deactivation of other neurotransmitters, namely transport into cells and intracellular hydrolysis. Even though anandamide and 2-AG are highly hydrophobic molecules, their penetration into neurons is likely to occur via a carrier-mediated transport system, rather than through passive diffusion. Thus, endocannabinoid accumulation in neurons is structurally specific, displays classical saturation kinetics and is selectively inhibited by drugs such as AM404 [18,23,24]. However, this putative transport system does not require cellular energy or external Na^+, implying that it might be mediated through a mechanism of facilitated diffusion [23,24].

Although anandamide and 2-AG share a functionally similar uptake mechanism, they follow two distinct routes of intracellular breakdown. Anandamide is metabolized by fatty acid amide hydrolase (FAAH), a membrane-bound intracellular serine hydrolase that also cleaves oleoyl-ethanolamine, an endogenous satiety factor [25,26], and other lipid amides. By contrast, the hydrolysis of 2-AG is probably catalyzed by monoglyceride lipase (MGL), a cytosolic serine hydrolase that converts 2- and 1-monoglycerides into fatty acid and glycerol [27].

FAAH as a drug target

In the rat brain, neurons that express FAAH are often found in the proximity of axon terminals containing CB_1 receptors, providing important evidence for a role of FAAH in anandamide deactivation [28]. Moreover, mutant mice lacking the gene encoding FAAH (*Faah*) cannot metabolize anandamide and show various signs of an exaggerated anandamide tone (for example, reduced pain sensation) [29]. These findings suggest that selective inhibitors of intracellular FAAH activity could enhance the actions of anandamide in brain areas where synthesis and release of this compound occur under physiological conditions. The increased anandamide tone produced by blocking FAAH might result in a more restricted spectrum of pharmacological effects than those produced by a direct-acting CB_1 agonist and, possibly, in fewer side effects. A similar paradigm has already been successful in pharmaceutical discovery: for example, inhibitors of serotonin reuptake and intracellular monoamine oxidase, which are clinically used in the treatment of depression, act by protecting biogenic amines from degradation and making them more available for receptor activation.

Can this concept be applied to the endocannabinoid system? To answer this question, appropriate pharmacological tools are needed. Although several FAAH inhibitors have been described, most of these do not meet the necessary criteria of potency, bioavailability or target selectivity (Table 1). Recently, however, a new class of small-molecule FAAH inhibitors was identified, which might obviate many of these limitations [30,31]. Lead compounds in this class, such as the substituted biphenyl carbamate URB597 (Figure 1), produce a rapid and persistent inhibition of brain FAAH activity both *in vitro* and *in vivo*. For example, URB597 inhibits FAAH activity with a half-maximal inhibitory concentration (IC_{50}) of 0.5 nM in primary cultures of rat brain neurons, and a half-maximal inhibitory dose (ID_{50}) of 0.15 mg kg^{-1} after parenteral administration in the rat. Importantly, URB597 is 7500 to 25 000 times more selective for FAAH than any other cannabinoid-related targets, including CB_1 and CB_2 receptors, endocannabinoid transport and MGL [30].

Although URB597 increases brain anandamide levels, it does not mimic the spectrum of pharmacological responses produced by typical CB_1 agonists. For example, parenteral doses of URB597 (0.3–1 mg kg^{-1}) that

Table 1. Fatty acid amide hydrolase (FAAH) inhibitors

Name	Description	Refs
Arachidonoyl trifluoromethyl ketone (ATFMK)	Reversible electrophilic carbonyl inhibitor. It belongs to the trifluoromethyl ketones, which, together with α-keto esters and α-keto amides, represent the three classes of anandamide-analog putative 'transition-state inhibitors'. ATFMK not only inhibits FAAH ($IC_{50} \approx 7.5~\mu M$) but also inhibits cytosolic phospholipase A2 (cPLA2) and binds to CB_1 receptors.	[42,43]
1-oxazolo[4,5-b]pyridin-2-phenylpentyl ketone	The most potent reversible FAAH inhibitor, tested *in vitro*, among several α-keto-oxazolopyridine derivatives ($IC_{50} \approx 0.2$ nM). Although the potency of this class of compounds is very high, their target selectivity and bioavailability have not been determined.	[44,45]
Palmitylsulfonyl fluoride (AM374)	This compound belongs to a family of sulfonyl fluorides, irreversible inhibitors of FAAH ($IC_{50} \approx 13$ nM) that also bind to the CB_1 receptor. It also inhibits *Escherichia coli* phospholipase A.	[46–48]
Hydro(pero)xy-anandamides	FAAH inhibitors of natural origin.	[49]
URB532 and URB597	These compounds belong to a series of alkylcarbamic acid aryl esters, designed as inhibitors of FAAH by progressive modification of the structure of the anti-cholinesterase agent carbaryl.	[30,31]
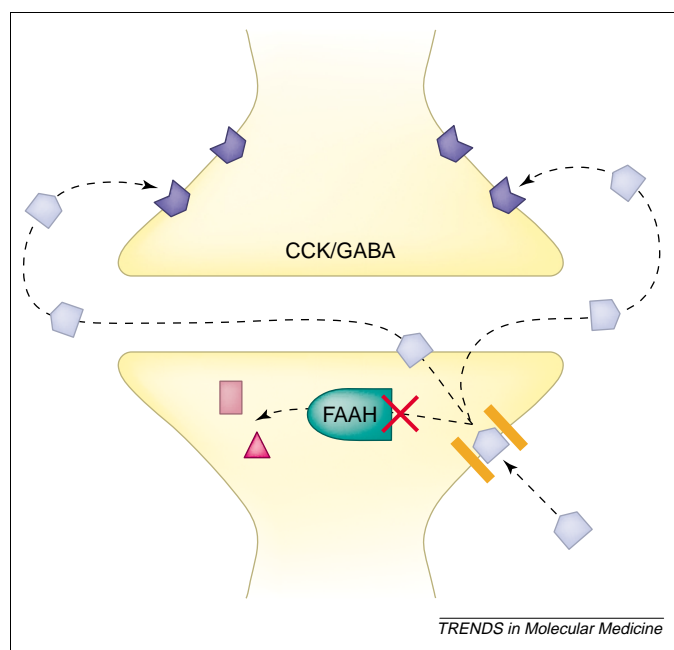
Figure 2. Effects of fatty acid amide hydrolase (FAAH) inhibitors on brain anandamide signaling. Anandamide is deactivated through a two-step process of transmembrane transport and intracellular hydrolysis. Transport is mediated by a hypothetical facilitated diffusion carrier system (endocannabinoid transporter; orange bars). Hydrolysis is catalyzed by a membrane-bound FAAH, which cleaves anandamide (light blue) to produce arachidonic acid (rose rectangle) and ethanolamine (pink triangle). According to this hypothetical model, pharmacological blockade of FAAH might cause unmetabolized anandamide to accumulate in and, eventually, leak out of neurons. This could result in the extracellular accumulation of anandamide and the increased local activation of CB_1 receptors (dark blue).

virtually eliminate brain FAAH activity do not produce catalepsy (rigid immobility), hypothermia or hyperphagia (increased food intake), three cardinal signs of cannabinoid intoxication in the rodent [2]. The compound exerts some degree of anti-nociception in the mouse hot plate test but this effect is very modest when compared with those of CB_1 agonists [30].

Despite its lack of typical cannabimimetic actions, URB597 elicits marked anxiolytic-like responses, which have been documented in two models of anxiety: the elevated zero maze test in adult rats and the isolation-induced ultrasonic vocalization test in rat pups [30].

The zero maze consists of an elevated annular platform divided into four quadrants, two open and two closed. The test is based on the conflict between an animal's instinct to explore its environment and its fear of open spaces, where it might be attacked by predators [32,33]. Anxiolytic agents such as the benzodiazepines increase the proportion of time spent in, and the number of entries made into, the open compartments. The FAAH inhibitor URB597 produces similar anxiolytic-like responses, with a maximal effect at a dose of $0.1 \, mg \, kg^{-1}$, which inhibits brain FAAH activity by $\sim 50\%$. As expected of an anandamide-mediated response, this effect is attenuated by the CB_1 antagonist rimonabant (SR141716A) at a dose at which the compound has no anxiogenic effect.

The ultrasonic vocalization emission test measures the number of stress-induced vocalizations emitted by rat pups removed from their nest [34–36]. URB597 markedly reduces these vocalizations at a dose of $0.1 \, mg \, kg^{-1}$, as observed with clinically used anxiolytic

and antidepressant drugs. The anxiolytic-like actions of URB597 are accompanied by a modest decrease in movement, which is observed, however, at doses that are ten times higher than those causing anxiolysis. Even at these high doses, URB597 exerts no overt cannabimimetic effects such as catalepsy or hypothermia [30].

Concluding remarks

How do FAAH inhibitors affect behavior? Moreover, why do they only produce a limited set of all possible cannabimimetic effects? Experiments *in vitro* show that URB597 causes unmetabolized anandamide to accumulate and, eventually, to leak out of brain neurons [30]. *In vivo*, this would result in an extracellular accumulation of anandamide at its sites of synthesis and, consequently, in an increased local activation of CB_1 receptors (Figure 2). If anandamide release in brain areas engaged in the processing of emotional information occurs at higher levels than in other structures, this might explain the restricted spectrum of action of URB597. That this might be the case is suggested by a recent study, which shows that anandamide content in the mouse amygdala rises when
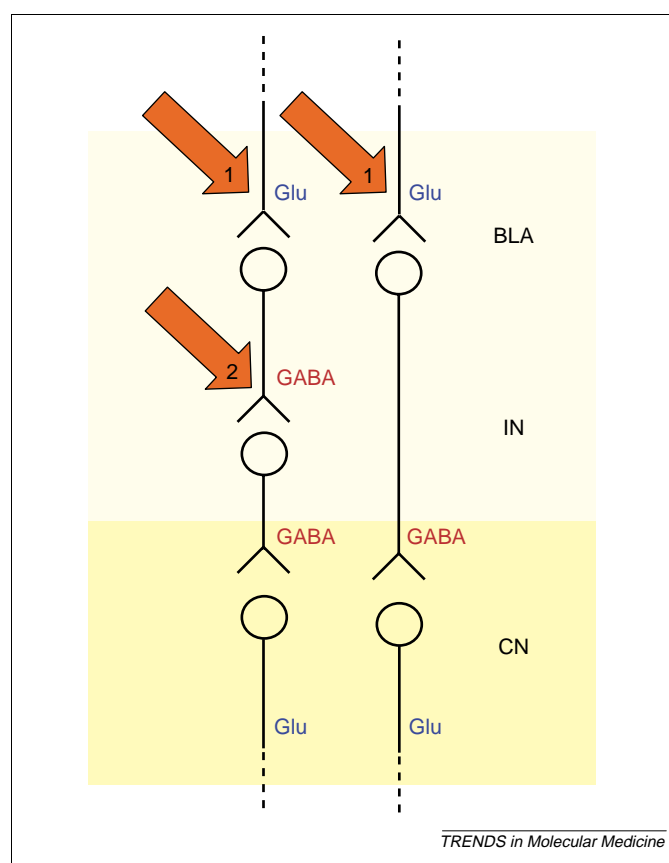
Figure 3. Possible sites of action of fatty acid amide hydrolase (FAAH) inhibitors in the amygdala. The enhanced anxiety that accompanies CB_1 receptor blockade and the anti-anxiety properties of FAAH inhibitors suggest that endocannabinoid substances could be generated in the amygdala during anxiety and that they might regulate emotional states by influencing amygdala outputs. This could be accomplished in several ways. By reducing γ-aminobutyric acid (GABA) release from CB_1-positive interneurons (arrow 2) in the basolateral amygdala (BLA), the endocannabinoids might disinhibit GABAergic cells in the adjacent intercalated nuclei (IN) and consequently inhibit pyramidal neurons in the central nucleus (CN), the primary efferent structure of the amygdala. In addition, the endocannabinoids might inhibit glutamate (Glu) release from cortically derived axon terminals (arrow 1) [50], which richly innervate the basolateral complex (for review, see [2]).

the animal is conditioned to expect a foot shock after hearing a tone [37]. This implies that the endocannabinoid system, and anandamide in particular, might be activated in response to anxiogenic situations such as that generated by a tone preceding a painful stimulus. The evidence reviewed above would further suggest that this activation could be part of a negative feedback system that limits anxiety.

The basolateral amygdala, the anterior cingulate cortex, the prefrontal cortex and the hippocampus are some of the possible brain structures implicated in these effects because they are directly involved in the regulation of emotional behavior [38] and contain high densities of CB_1 receptors [13,39]. Cannabinoid agonists have a profound impact on GABAergic and glutamatergic transmission in these regions [2], suggesting that both neurotransmitter systems could be involved in the anxiolytic effects of FAAH inhibitors (Figure 3). However, they need not be the only ones. Throughout the forebrain, CB_1-positive GABAergic interneurons express the neuropeptide cholecystokinin-8 (CCK-8) [2], the anxiogenic roles of which are well documented [40]. CB_1 agonists inhibit K^+-evoked CCK-8 release in the hippocampus [41], suggesting that interactions between anandamide and CCK-8 might also contribute to the regulation of emotional states.

Acknowledgements

References

1 Gorman, J.M. (2003) New molecular targets for antianxiety interventions. *J. Clin. Psychiatry* 64, 28–35

2 Freund, T.F. *et al.* (2003) Role of endogenous cannabinoids in synaptic signaling. *Physiol. Rev.* 83, 1017–1066

3 Tournier, M. *et al.* (2003) Cannabis use and anxiety in daily life: a naturalistic investigation in a non-clinical population. *Psychiatry Res.* 118, 1–8

4 Hall, W. and Solowij, N. (1998) Adverse effects of cannabis. *Lancet* 352, 1611–1616

5 Wachtel, S.R. *et al.* (2002) Comparison of the subjective effects of Δ(9)-tetrahydrocannabinol and marijuana in humans. *Psychopharmacology (Berl.)* 161, 331–339

6 Arévalo, C. *et al.* (2001) Cannabinoid effects on anxiety-related behaviours and hypothalamic neurotransmitters. *Pharmacol. Biochem. Behav.* 70, 123–131

7 Zuardi, A. *et al.* (1982) Action of cannabidiol on the anxiety and other effects produced by Δ9-THC in normal subjects. *Psychopharmacology (Berl.)* 76, 245–250

8 Onaivi, E.S. *et al.* (1995) Neurobehavioral effects of Δ9-THC and cannabinoid (CB1) receptor gene expression in mice. *Behav. Brain Res.* 72, 115–125

9 Chakrabarti, A. *et al.* (1998) Neurobehavioral effects of anandamide and cannabinoid receptor gene expression in mice. *Brain Res. Bull.* 45, 67–74

10 Berrendero, F. and Maldonado, R. (2002) Involvement of the opioid system in the anxiolytic-like effects induced by Δ(9)-tetrahydrocannabinol. *Psychopharmacology (Berl.)* 163, 111–117

11 Sulcova, E. *et al.* (1998) Biphasic effects of anandamide. *Pharmacol. Biochem. Behav.* 59, 347–352

12 Rodríguez de Fonseca, F. *et al.* (1996) Corticotropin-releasing factor (CRF) antagonist [D-Phe12,Nle21,38,C alpha MeLeu37]CRF attenuates the acute actions of the highly potent cannabinoid receptor agonist HU-210 on defensive-withdrawal behavior in rats. *J. Pharmacol. Exp. Ther.* 276, 56–64

13 Herkenham, M. *et al.* (1990) Cannabinoid receptor localization in brain. *Proc. Natl. Acad. Sci. U. S. A.* 87, 1932–1936

14 Hájos, N. *et al.* (2001) Novel cannabinoid-sensitive receptor mediates inhibition of glutamatergic synaptic transmission in the hippocampus. *Neuroscience* 106, 1–4

15 Haller, J. *et al.* (2002) The effects of genetic and pharmacological blockade of the CB1 cannabinoid receptor on anxiety. *Eur. J. Neurosci.* 16, 1395–1398

16 Navarro, M. *et al.* (1997) Acute administration of the CB1 cannabinoid receptor antagonist SR 141716A induces anxiety-like responses in the rat. *Neuroreport* 8, 491–496

17 Rodgers, R.J. *et al.* (2003) 'One-trial sensitization' to the anxiolytic-like effects of cannabinoid receptor antagonist SR141716A in the mouse elevated plus-maze. *Eur. J. Neurosci.* 17, 1279–1286

18 Di Marzo, V. *et al.* (1994) Formation and inactivation of endogenous cannabinoid anandamide in central neurons. *Nature* 372, 686–691

19 Stella, N. *et al.* (1997) A second endogenous cannabinoid that modulates long-term potentiation. *Nature* 388, 773–778

20 Wilson, R.I. and Nicoll, R.A. (2001) Endogenous cannabinoids mediate retrograde signalling at hippocampal synapses. *Nature* 410, 588–592

21 Ohno-Shosaku, T. *et al.* (2001) Endogenous cannabinoids mediate retrograde signals from depolarized postsynaptic neurons to presynaptic terminals. *Neuron* 29, 729–738

22 Kreitzer, A. and Regehr, W.G. (2001) Retrograde inhibition of presynaptic calcium influx by endogenous cannabinoids at excitatory synapses onto Purkinje cells. *Neuron* 29, 717–727

23 Beltramo, M. *et al.* (1997) Functional role of high-affinity anandamide transport, as revealed by selective inhibition. *Science* 277, 1094–1097

24 Hillard, C.J. *et al.* (1997) Accumulation of N-arachidonoylethanolamine (anandamide) into cerebellar granule cells occurs via facilitated diffusion. *J. Neurochem.* 69, 631–638

25 Rodríguez de Fonseca, F. *et al.* (2001) An anorexic lipid mediator regulated by feeding. *Nature* 414, 209–212

26 Fu, J. *et al.* (2003) Oleylethanolamide regulates feeding and body weight through activation of PPAR-α. *Nature* 425, 90–93

27 Dinh, T.P. *et al.* (2002) Brain monoglyceride lipase participating in endocannabinoid inactivation. *Proc. Natl. Acad. Sci. U. S. A.* 99, 10819–10824

28 Egertova, M. *et al.* (2003) Comparative analysis of fatty acid amide hydrolase and CB1 cannabinoid receptor expression in the mouse brain: evidence of a widespread role for fatty acid amide hydrolase in regulation of endocannabinoid signaling. *Neuroscience* 119, 481–496

29 Cravatt, B.F. *et al.* (2001) Supersensitivity to anandamide and enhanced endogenous cannabinoid signaling in mice lacking fatty acid amide hydrolase. *Proc. Natl. Acad. Sci. U. S. A.* 98, 9371–9376

30 Kathuria, S. *et al.* (2003) Modulation of anxiety through blockade of anandamide hydrolyis. *Nat. Med.* 9, 76–81

31 Tarzia, G. *et al.* (2003) Design, synthesis, and structure-activity relationships of alkylcarbamic acid aryl esters, a new class of fatty acid amide hydrolase inhibitors. *J. Med. Chem.* 46, 2352–2360

32 Bickerdike, M.J. *et al.* (1994) The influence of 5-hydroxytryptamine re-uptake blockade on CCK receptor antagonist effects in the rat elevated zero-maze. *Eur. J. Pharmacol.* 271, 403–411

33 Shepherd, J.K. *et al.* (1994) Behavioural and pharmacological characterisation of the elevated 'zero- maze' as an animal model of anxiety. *Psychopharmacology (Berl.)* 116, 56–64

34 Insel, T.R. *et al.* (1986) Rat pup ultrasonic isolation calls: possible mediation by the benzodiazepine receptor complex. *Pharmacol. Biochem. Behav.* 24, 1263–1267

35 Miczek, K.A. *et al.* (1995) Aggression, anxiety and vocalizations in animals: GABAA and 5-HT anxiolytics. *Psychopharmacology (Berl.)* 121, 38–56

36 Winslow, J.T. and Insel, T.R. (1991) Infant rat separation is a sensitive test for novel anxiolytics. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 15, 745–757

37 Marsicano, G. *et al.* (2002) The endogenous cannabinoid system controls extinction of aversive memories. *Nature* 418, 530–534

38 Cahill, L. and McGaugh, J.L. (1998) Mechanisms of emotional arousal and lasting declarative memory. *Trends Neurosci.* 21, 294–299

39 Glass, M. *et al.* (1997) Cannabinoid receptors in the human brain: a detailed anatomical and quantitative autoradiographic study in the fetal, neonatal and adult human brain. *Neuroscience* 77, 299–318

40 Rotzinger, S. and Vaccarino, F.J. (2003) Cholecystokinin receptor subtypes: role in the modulation of anxiety-related and reward-related behaviours in animal models. *J. Psychiatry Neurosci.* 28, 171–181

41 Beinfeld, M.C. and Connolly, K. (2001) Activation of CB1 cannabinoid receptors in rat hippocampal slices inhibits potassium-evoked cholecystokinin release, a possible mechanism contributing to the spatial memory defects produced by cannabinoids. *Neurosci. Lett.* 301, 69–71

42 Koutek, B. *et al.* (1994) Inhibitors of arachidonoyl ethanolamide hydrolysis. *J. Biol. Chem.* 269, 22937–22940

43 Riendeau, D. *et al.* (1994) Arachidonyl trifluoromethyl ketone, a potent inhibitor of 85-kDa phospholipase A2, blocks production of arachidonate and 12-hydroxyeicosatetraenoic acid by calcium ionophore-challenged platelets. *J. Biol. Chem.* 269, 15619–15624

44 Boger, D.L. *et al.* (2000) Exceptionally potent inhibitors of fatty acid amide hydrolase: the enzyme responsible for degradation of endogenous oleamide and anandamide. *Proc. Natl. Acad. Sci. U. S. A.* 97, 5044–5049

45 Boger, D.L. *et al.* (2001) α-keto heterocycle inhibitors of fatty acid amide hydrolase: carbonyl group modification and α-substitution. *Bioorg. Med. Chem. Lett.* 11, 1517–1520

46 Deutsch, D.G. *et al.* (1997) Fatty acid sulphonyl fluorides inhibit anandamide metabolism and bind to the cannabinoid receptor. *Biochem. Biophys. Res. Commun.* 231, 217–221

47 Horrevoets, J. *et al.* (1991) Inactivation of *Escherichia coli* outer-membrane phospholipase A by the affinity label hexadecanesulfonyl fluoride. Evidence for an active-site serine. *Eur. J. Biochem.* 198, 247–253

48 Horrevoets, A.J. (1991) Activation of reconstituted *Escherichia coli* outer-membrane phospholipase A by membrane-perturbing peptides results in an increased reactivity towards the affinity label hexadecanesulfonyl fluoride. *Eur. J. Biochem.* 198, 255–261

49 van der Stelt, M. *et al.* (2002) Oxygenated metabolites of anandamide and 2-arachidonoylglycerol: conformational analysis and interaction with cannabinoid receptors, membrane transporter, and fatty acid amide hydrolase. *J. Med. Chem.* 45, 3709–3720

50 Azad, S.C. *et al.* (2003) Activation of the cannabinoid receptor type 1 decreases glutamatergic and GABAergic synaptic transmission in the lateral amygdala of the mouse. *Learn. Mem.* 10, 116–128